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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Flechtner et al.	Confirmation No.:	4151
Serial No.:	10/776,521	Art Unit:	1648
Filed:	February 12, 2004	Examiner:	Blumel, Benjamin P.
For:	HEAT SHOCK PROTEIN-BASED VACCINES AND IMMUNOTHERAPIES	Attorney Docket No:	8449-405-999

DECLARATION OF THE INVENTORS UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, Paul Slusarewicz and Sunil Mehta, do declare that:

1. Paul Slusarewicz (i) is a citizen of the United Kingdom residing at 13847 Crown Bluff, San Antonio, Texas; (ii) currently holds the position of Manager, DFB Central Research at DFB Pharmaceuticals, Inc., having a place of business in San Antonio, Texas; (iii) received a Ph.D. degree in Biochemistry from Imperial Cancer Research Fund and University College, University of London and a B.S. degree in Biochemistry from Imperial College of Science, Technology and Medicine, University of London; and (iv) was an employee of Mojave Therapeutics, Inc. ("Mojave") from 2001 to 2003. Paul Slusarewicz's academic and technical experience and honors, and a list of his publications are set forth in the *curriculum vitae*, attached hereto as Exhibit 1.

2. Sunil Mehta (i) is a citizen of India residing in Durham, North Carolina; (ii) currently holds the position of Associate Director, Fermentation & Cell Culture at KBI Biopharma Inc., having a place of business in Durham, North Carolina; (iii) received a Ph.D. degree in Molecular Pharmacology from Brown University; and (iv) was an

employee of Mojave from 2001 to 2003. Sunil Mehta's academic and technical experience and honors are set forth in the *curriculum vitae*, attached hereto as Exhibit 2.

3. We are co-inventors of the invention described and claimed in the above-identified application, United States ("U.S.") Patent Application No. 10/776,521 ("the '521 application") entitled "Heat Shock Protein-Based Vaccines and Immunotherapies" and filed on February 14, 2004.

4. We understand that in July 2004, the intellectual property assets of Mojave, the original Assignee of the '521 application, were acquired by Antigenics, Inc. ("Antigenics"), which has a place of business in Lexington, Massachusetts, and that Antigenics is the present owner of the entire right, title and interest in, to and under the invention described and claimed in the '521 application.

5. The claims of the '521 application are directed to a hybrid antigen comprising at least one antigenic domain of an infectious agent or tumor antigen and a binding domain that non-covalently binds to a heat shock protein, optionally with a peptide linker separating the antigenic domain and binding domain, wherein the binding domain comprises the amino acid sequence Asn Leu Leu Arg Leu Thr Gly Trp ("NLLRLTGW"), Phe Tyr Gln Leu Ala Leu Thr Trp ("FYQLALTW"), or Arg Lys Leu Phe Phe Asn Leu Arg Trp ("RKLFFNLRW"); compositions comprising at least one such hybrid antigen and a pharmaceutically acceptable carrier; compositions comprising a non-covalent complex of at least one such hybrid antigen and at least one heat shock protein; and methods of inducing an immune response to a tumor antigen or an infectious agent or methods of treating an infectious disease or cancer comprising administering at least one such hybrid antigen, optionally non-covalently bound to at least one heat shock protein.

6. We have reviewed and understand the '521 application. We also have reviewed and understand portions of the reference Wieland et al., U.S. Application

Publication No. US 2004/0071656 A1 ("Wieland et al.") including those paragraphs of Wieland et al. referenced in Paragraph 7 below. Wieland et al. is the publication of U.S. Application No. 10/328,953 ("the Wieland application") filed on December 23, 2002. We understand that in an Office Action dated September 21, 2006, the Examiner has rejected the claims of the '521 application under consideration as lacking novelty in view of Wieland et al. We are informed that the Wieland application claims benefit of provisional applications filed on December 26, 2001, December 27, 2001, April 12, 2002, July 29, 2002, and September 28, 2002, respectively.

7. Wieland et al. discloses a complex of a heat shock protein and a hybrid antigen, the hybrid antigen comprising an antigenic domain, a heat shock protein binding domain, and optionally a short peptide linker interposed therebetween (*see* Wieland et al. at ¶20). In particular, the heat shock protein binding domain can comprise the amino acid sequence NLLRLTGW, FYQLALTW, or RKLFFNLRW (*see* Wieland et al. at ¶¶129-130).

8. The invention described and claimed in the '521 application was conceived by us prior to December 26, 2001, as evidenced by the facts presented in Paragraphs 9-14 below.

9. Attached hereto as Exhibits 3-6 are copies of documents dated prior to December 26, 2001 that document our conception of the invention of a hybrid antigen containing the amino acid sequence NLLRLTGW, FYQLALTW, or RKLFFNLRW, optionally containing a peptide linker, and optionally complexed non-covalently to a heat shock protein, as originally conceived by us and claimed in the '521 application. We have reviewed each of the documents of Exhibits 3-6.

10. Attached as Exhibit 3 are documents entitled "Certificate of Analysis." Although the date on the pages of Exhibit 3 has been blanked-out, such date is prior to December 26, 2001. The documents of Exhibit 3 disclose the amino acid sequences

NLLRLTGW, FYQLALTW, and RKLFFNLRW of the heat shock protein binding peptides designated SJAV1, SJAV2, and SJAV3, respectively. These peptides were ordered for members of our research group from New England Peptide, Inc. The documents contain quality control test results for each of the SJAV1 (NLLRLTGW), SJAV2 (FYQLALTW), and SJAV3 (RKLFFNLRW) peptides supplied by New England Peptide, Inc.

11. Exhibit 4 contains notebook pages 57-58 of Notebook No. 44 issued to Irina Kostareva. Although the date on the pages of Exhibit 4 has been blanked-out, such date is prior to December 26, 2001. Exhibit 4 documents the conjugation of the antigen ovalbumin ("OVA") to the heat shock protein binding domain SJAV1 (NLLRLTGW) peptide or "Jav-G-S-G" peptide to generate a hybrid antigen. The Jav-G-S-G peptide has a heat shock protein binding domain followed by the GSG peptide linker. Irina Kostareva was an employee of Mojave, who reported to Paul Slusarewicz. The experiments documented in the notebook pages of Exhibit 4 were carried out by Irina Kostareva under the supervision of Paul Slusarewicz.

12. As shown in Exhibit 4, OVA was dissolved in water and activated for covalent conjugation with DSS (Di(N-succinimidyl) suberate). Subsequently, the activated OVA was incubated with the SJAV1 (NLLRLTGW) or Jav-G-S-G peptide for 1 hour and 30 minutes to facilitate covalent conjugation. The reaction was stopped by addition of glycine, and the resulting conjugates were purified.

13. Exhibit 5 contains notebook pages 73-75 of Notebook No. 44 issued to Irina Kostareva. Although the date on the pages of Exhibit 5 has been blanked-out, such date is prior to December 26, 2001. Exhibit 5 documents the conjugation of OVA to the heat shock protein binding domain SJAV2 (FYQLALTW) peptide (referred to as "SJ2" in Exhibit 5). The experiments documented in the notebook pages of Exhibit 5 were carried out by Irina Kostareva under the supervision of Paul Slusarewicz.

14. As shown in Exhibit 5, OVA was activated for covalent conjugation with DSP (Dithiobis (succinimidyl propionate)), reduced with DTT (dithiothreitol), and purified before chemical conjugation to the SJAV2 peptide. The SJAV2 peptide was activated with SMPH (Succinimidyl-6-(β-maleimidopropionamido)hexanoate). Subsequently, the activated OVA was incubated with the SJAV2 peptide overnight at 4°C to facilitate covalent conjugation.

15. The experiments described in Paragraphs 11-14 above were carried out in the United States at the laboratory facilities of Mojave in Hawthorne, New York prior to December 26, 2001.

16. A copy of an e-mail from Sunil Mehta to Michael A. Yamin, then Director of Intellectual Property and Licensing at Mojave, is enclosed hereto as Exhibit 6. Although the date of the e-mail has been blanked-out, such date is prior to December 26, 2001. This e-mail documents the disclosure of our invention to Michael A. Yamin for purposes of preparing U.S. Provisional Application No. 60/447,142 filed on February 13, 2003 and subsequently the '521 application and its other priority applications. The e-mail discloses the amino acid sequences, dissociation constants ("Kds") for binding to Hsp70, heat shock proteins the peptides are disclosed to bind to (in the respective listed publications), and other related information for the peptides S-Jav1 (NLLRLTGW), S-Jav2 (FYQLALTW), and S-Jav3 (RKLFFNLRW), which were conceived by us for use in generating hybrid antigens. The name designation for the peptides in Exhibit 6 is consistent with the names of the peptides referred to in Exhibits 3-5.[1]

17. As evidenced by Exhibit 6, the amino acid sequences NLLRLTGW, FYQLALTW, and RKLFFNLRW as heat shock protein binding peptides for use in covalent conjugation, optionally via a peptide linker, to an antigen to generate a hybrid antigen,

[1] Exhibit 5 refers to the S-Jav2 peptide as "SJ2."

optionally complexed non-covalently to heat shock protein, as well as the uses of such hybrid antigens and complexes in methods of inducing an immune response to a tumor antigen or an infectious agent or methods of treating an infectious disease or cancer, as originally conceived by us, were disclosed to Michael A. Yamin at a time prior to December 26, 2001.

18. Insofar as the claimed invention of our pending application, the '521 application, is suggested or disclosed by anything contained in Wieland et al., such invention was originally conceived by us and disclosed to Michael A. Yamin prior to December 26, 2001.

19. We declare further that all statements made in this Declaration of our knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: _____

Paul Slusarewicz, Ph.D.

Date: 15 JAN 08

Sunil Mehta, Ph.D.

Attachments:

Exhibit 1: *Curriculum Vitae* of Paul Slusarewicz, Ph.D.
Exhibit 2: *Curriculum Vitae* of Sunil Mehta, Ph.D.
Exhibit 3: Documents entitled "Certificate of Analysis"
Exhibit 4: Notebook Pages 57-58 of Notebook No. 44 Issued to Irina Kostareva
Exhibit 5: Notebook Pages 73-75 of Notebook No. 44 Issued to Irina Kostareva
Exhibit 6: E-mail from Sunil Mehta to Michael A. Yamin

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Tel: 210-402-0048 E-mail: paulslusarewicz@hotmail.com

Paul Slusarewicz

Experienced and Successful Biochemist, Cell Biologist and R&D Manager

Over ten years of international industrial R&D experience • U.S. Permanent Resident with over eight years of management experience in large and small corporate environments • Familiarity and experience with numerous business functions, including BD, IP, QA/QC and Marketing • Strong leader with excellent communication and presentation skills • Experienced at project planning and managing multiple projects • Exceptional intellectual and technical ability • Strong record of independently developing and executing successful R&D projects and programs aligned to corporate objectives

- Built numerous highly productive biochemistry teams and successful R&D programs in areas such as Protein Chemistry, Cell Biology, Dermatology, Wound Healing and Vaccine Development
- Represented Company's contract Bioanalytical capabilities to potential clients resulting in generation of new business opportunities
- Developed and transferred numerous analytical release tests for clinical formulations and raw materials
- Developed and refined protein purification processes, resulting in potential savings of hundreds of thousands of dollars in raw materials costs
- Developed claim support, PR and Q&A packages to support the marketing of a major home and personal care product brand
- Graduate with First Class Honours from Imperial College, London, consistently ranked by The Times as the U.K.'s number one Science and Engineering University and former E.M.B.O. Fellow, ranked in the top ten of all applicants of 1994 by the European Molecular Biology Organization

PROFESSIONAL EXPERIENCE

DFB PHARMACEUTICALS, INC., SAN ANTONIO, TEXAS **2003-2007**

Manager, DFB Central Research (2005-2007)

- Appointed Manager of newly formed Corporate Central Research division
- Developed successful research program in the areas of Dermatology and Wound Healing, already leading to the discovery of one lead drug candidate
- Managed three groups in the areas of Topical Formulation and Delivery Systems Development, Biochemistry and Cell Biology
- Member of several due diligence teams investigating possible product and corporate acquisitions

Manager, Biopharmaceutical Services, DPT Laboratories (2003 – 2004)

- Developed strong Bioanalytical group allowing the company to offer contract customers product development services for topical biological pharmaceuticals and represented the company to potential new clients, resulting in new $500,000 business opportunity
- Developed analytical assays for release and stability testing of new products and successfully transferred them to QC
- Developed and validated improved analytical method for API in currently marketed products which is being submitted to USP to supplant current USP method
- Supported current marketed products by overseeing new work requested by Marketing, improving existing formulations and troubleshooting OOS results obtained by QC

- Managed all early-stage development projects for product prototypes (i.e. up to 3 months of informal stability)
- Identified new biological API for next generation product. Currently working on IP filing and negotiating with prospective GMP manufacturers before transferring project into development

MOJAVE THERAPEUTICS, INC., HAWTHORNE, NY **2001-2003**

Group Leader, Protein Biochemistry

- Rapidly built a highly motivated and productive team from two to nine individuals and developed a successful R&D program leading to a step-change in the Company's understanding of the protein-protein interactions that occur with its vaccine formulations
- Redesigned Company's antigen delivery system, leading to an order-of-magnitude improvement in immune responses from vaccine formulations
- Developed qualified analytical assays to address FDA product characterization concerns, resulting in the lift of an FDA clinical hold
- Developed several receptor-ligand binding assays, including radio-ligand and fluorescence anisotropy, to characterize the interaction of various proteins of interest to the Company
- Developed and refined purification process for the Company's lead protein therapeutic, potentially introducing significant cost savings and developed purification processes for the in-house production of other endotoxin-free biologics of interest to the Company
- Developed equations and computer-based algorithms to predict the interaction of protein components in multivalent formulations as an aid to rational product design
- Liaised with Immunology, IP, QA, Regulatory and Business Development Departments in order to ensure alignment of R&D activities with corporate goals and milestones
- Several patents filed based on the above work

UNILEVER PLC, BEDFORD, ENGLAND **1996-2001**

Project Manager and Team Leader (2000 – 2001)

- In addition to the position of Hair Follicle Biology Team Leader was made responsible for the scientific development of the research program for a major new project
- This involved defining project scope with the business, developing and implementing a project plan, co-ordinating efforts of three laboratories

Product Development and Marketing Manager (2000)

- Sent on an eight-month secondment to Marketing and Development at Elida Faberge, Paris
- Developed a process to improve product stability and liased with production personnel to facilitate its transfer to Manufacturing
- Led the development of a PR package to support marketing and launch of a major global brand
- Verified technical product claims by liaising with Marketing, Legal and Research

Team Leader (1998 – 2000)

- Appointed leader of the six-person Hair Follicle Biology Group
- Discovered and purified novel enzyme isoforms specific to hair follicles
- Supervised multiple projects in areas such as cell biology, oxidative metabolism, enzyme kinetics and analytical assay development
- Consistently ensured delivery of the long-term research program and several and short-term projects to support marketing activities, often to very tight deadlines
- Appointed as chief research contact to the Latin American business region and primary liaison with major academic collaborators at the University of Cambridge

Research Scientist (1996 – 1998)

- Undertook two independent projects in the areas of cell biology and enzymology, resulting in the filing of four patents and in the attraction of new internal funding
- Disproved a widely held hypothesis in hair follicle biology research and developed alternative hypotheses with corroborating data

DARTMOUTH MEDICAL SCHOOL, HANOVER, NH **1995-1996**

Post-doctoral Fellow

- Purified and characterized a cytosolic protein that catalyses yeast vacuole fusion using numerous chromatographic and bulk-phase methods
- Purified untagged recombinant proteins by developing and scaling up new purification processes and also affinity tagged proteins using established protocols
- Developed fluorescent microscopic assays to probe the involvement of actin filaments and microtubules in the process of vacuole inheritance

IMPERIAL CANCER RESEARCH FUND, LONDON, ENGLAND **1990-1994**

Post-Graduate Research Student

- Purified various soluble and membrane-associated proteins and studied their enzymology
- Identified and purified a cytosolic proteinaceous matrix and developed binding assays to analyze its interaction with its ligands
- Raised polyclonal antibodies to the matrix that subsequently proved central to the cloning of a novel protein that plays a central role in membrane traffic in the Golgi apparatus
- Developed numerous enzyme assays and conducted analysed kinetic and inhibitor studies

EDUCATION

Ph.D. (Biochemistry). Imperial Cancer Research Fund and University College, University of London. Thesis title: 'Isolation of an intercisternal matrix which binds *medial*-Golgi enzymes.' 1994.

B.Sc. (Biochemistry). First Class Honours. Imperial College of Science, Technology and Medicine, University of London. 1990.

COMMUNICATION SKILLS

Highly proficient at presenting, both in the academic and industrial environment, and at all levels of seniority. Particularly adept in the use of graphic design applications to produce polished and professional presentations. Experienced at working in cross-functional teams and at communicating scientific concepts to laypersons. Excellent writing skills both in the technical and business context. Fluent in both English and Polish.

COMPUTER SKILLS

Proficient at using both PC and Macintosh platforms. Fully conversant with all major packages such as Word, Excel, MS Project, PowerPoint, Illustrator and Photoshop. Was responsible for maintenance of laboratory computers during Ph.D. and academic post-doc.

RELEVANT COURSES

Two-Day Leadership Training Course • Three-Day Business Awareness Course • Three-Day Project Planning Training • One-Week People Management Course for Project Managers • Advanced Presentation Skills • Clinical Trial Organization/Good Clinical Practice Course • Safety Awareness for Line Managers

PUBLICATIONS

Blount M.A., Goff S. and **Slusarewicz, P.** *In vitro* degradation of the inner root sheath in human hair follicles lacking sebaceous glands. Manuscript submitted.

Andjelic, S., Musselli, C., Mayhew, M., Warren, N., Reid, M., **Slusarewicz, P.**, Houghton, A.N., Livingston, P.O. and Barber, B. JAVELIN^{TM}- fusion peptides bound to HSP70: a candidate vaccine for melanoma. Manuscript in preparation.

Telgenhoff, D., Lam, K., Ramsay, S., Vasquez, V., Posadas, K., **Slusarewicz, P.**, Attar, P., and Shroot, B. The influence of papain urea copper chlorophyllin on wound matrix remodelling. Manuscript submitted.

Flechtner, J.B., Prince-Cohane, K., Mehta, S., **Slusarewicz, P.**, Kays-Leonard, A., Barber, B.H., Levey, D.L. and Andjelic, S. (2006). High affinity interactions between peptides and heat shock protein 70 augment $CD8^+$ T lymphocyte immune responses. *J. Immunol.*177: 1017-1027

Slusarewicz, P., Xu, Z., Seefeld, K., Haas, A. and Wickner, W.T. (1997). $I^B{}_2$ is a small cytosolic protein that participates in vacuole fusion. *Proc. Natl. Acad. Sci. USA* 94: 5582-5587.

Nakamura, N., Rabouille, C., Watson, R., Nilsson, T., Hui, N., **Slusarewicz, P.**, Kreis, T. and Warren, G. (1996). Characterisation of a *cis*-Golgi matrix protein, GM130. *J. Cell Biol.* 131: 1715-1726

Hoe, M.H., **Slusarewicz, P.**, Watson, R., Misteli,T. and Warren, G. (1995). Evidence for recycling of the resident *medial/trans* Golgi enzyme, N-acetylglucosaminyltransferase I, in ldlD cells. *J. Biol. Chem.* 270: 25057-25063.

Slusarewicz, P., and Warren, G. (1995). 1-Deoxymannojirimycin is a non-competitive inhibitor of mannosidase II. *Glycobiology* 5: 154-155.

Nilsson, T., Hoe, M.H., **Slusarewicz, P.**, Rabouille, C., Watson, R., Hunte, F., Watzele, G., Berger, E.G., and Warren, G. (1993). Kin recognition between *medial*-Golgi enzymes in HeLa cells. *EMBO J.* 13: 562-574.

Slusarewicz, P., Nilsson, T., Hui, N., Watson, R., and Warren, G. (1993). Isolation of a matrix that binds *medial*-Golgi enzymes. *J. Cell Biol.* 124: 405-413.

Nilsson, T., **Slusarewicz, P.**, Hoe, M.H., and Warren, G. (1993). Kin recognition. A model for the retention of Golgi enzymes. *FEBS Lett.* 330:1-4.

Nilsson, T., Pypaert, M., Hoe, M.H., **Slusarewicz, P.**, Berger, E.G., and Warren, G. (1993). Overlapping distributions of two glycosyltransferases in the Golgi apparatus of HeLa cells. *J. Cell Biol.* 120:5-13.

PATENTS

Fletchner, J., Prince-Cohane, K., Mehta, S. **Slusarewicz, P.**, Andjelic, S. and Barber, B. (2004). Improved heat shock protein-based vaccines and immunotherapies. WO2004071457. US2005202033. EP1622563. CA2520591. AU2004212003.

Fletchner, J., Prince-Cohane, K., Mehta, S. **Slusarewicz, P.**, Andjelic, S. and Barber, B. (2004). Improved heat shock protein-based vaccines and immunotherapies. WO2004/091493. US2005214312.

Slusarewicz, P. (2003). Hair colouring compositions. *US2003037385. WO02080867.*

Slusarewicz, P. (2002). Method of colouring hair. *US2002197224. WO02080868.*

Blount, M.A., Pearce, M.L., Jackson, K., Noel, N., Davis, M.A, and **Slusarewicz, P.** (2002). Treating hair by targeting enzymes. *US2002119112. US2001031250. US6399052. EP117108. AU3818300. WO0064405.*

Parmar, P., Beck, J.S., Davis, M.A., **Slusarewicz, P.,** and Westgate, G.E. (2002). Hair treatment composition, method of use. *US2002031483. US2002031483. EP1115369. AU5974999. BR9913971.*

POSTERS, ARTICLES AND INVITED PRESENTATIONS

Kulkarni, V., Pillai, R., Shah, B., Jaime, M., **Slusarewicz, P.,** Cargill, I. and Shroot, B.. Multivesicular emulsions as a versatile topical drug delivery system. 63rd AAPS Meeting, San Antonio, Texas. October, 2006.

Westgate, G.E., Blount M.A., Parmar, P., **Slusarewicz, P.,** and Goff S. Degradation of the Hair Follicle Inner Root Sheath. 12th European Hair Research Society Meeting. London. June, 2006.

Shi, L., Lam, K., Cowart, J., **Slusarewicz, P.,** Attar, P. and Shroot, B. Correlation of *in vitro* and *in vivo* efficacy evaluations of papain wound debriding agents. 16th Wound Healing Society Meeting. Scottsdale, Arizona. May, 2004.

Shi, L., Keen, J., **Slusarewicz, P.** and Shroot, B .A novel model for assessment of enzymatic debriding agents. 14th Wound Healing Society Meeting. Atlanta, Georgia. May, 2004.

Baker, J., Prince, K., Mehta, S., **Slusarewicz, P.,** Barber, B. and Andjelic, S. Higher affinity interaction between MHC class I epitope and HSP70 augments CD8+ T lymphocyte immune responses. Keystone Symposium, Basic Aspects of Tumor Immunobiology, Keystone, Colorado. February, 2003.

Barber, B., Andjelic, S., Musselli, C., Mayhew, M., Warren, N., Reid, M., **Slusarewicz, P.,** Houghton, A.N. and Livingston, P.O. Analysis of T-cell responses following vaccination with hsp70-Javelin peptide complexes: A Phase I clinical trial in melanoma patients. 3rd International Conference Heat shock proteins in Immune Response. Farmington, Connecticut. October, 2002.

Slusarewicz, P. Talk Title: 'Degradation of the human hair follicle inner root sheath *in vitro*.' British Skin Club Meeting, University of Sheffield. December 2000.

Slusarewicz, P. Talk title: 'Degradation of the hair follicle inner root sheath.' 4th International Cosmetics Symposium, Istanbul. June 2000.

Hui, N., Nakamura, N., **Slusarewicz, P.** and Warren, G. Purification of rat liver Golgi stacks. *In* Cell Biology: A laboratory handbook, 2nd edition. Celis, J.E., editor. 1997. Academic Press, Inc., Orlando, Florida.

Hui, N., **Slusarewicz, P.,** Nakamura, N., Warren, G. and Nilsson, T. (1995). Isolation of a Golgi Oligomer. 43rd ASCB Meeting. San Franscisco, California. December, 1995.

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Slusarewicz, P. Talk title: 'Identification of an intercisternal matrix which binds *medial*-Golgi enzymes'. 33rd A.S.C.B. Meeting, New Orleans. December 1993.

REFERENCES

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EXPERIENCE

CENTOCOR RESEARCH & DEVELOPMENT, INC. (A wholly owned subsidiary of Johnson and Johnson), Pharmaceutical Development, Radnor, PA

Senior Research Scientist, 2007-Present

- Led a team to develop stable CHO cell lines producing high titers of therapeutic antibodies in fed-batch cultures.

Research Scientist, 2004-2006

- Developed a simple, economical, and GMP scalable system to produce therapeutic ScFv in *E.coli* for phase I/II studies.
- Successfully expressed correctly folded proteins with multiple disulfide bonds in *E.coli* and scaled up production using fermentation.
- Increased recombinant antibody production from mammalian cells by use of inexpensive small molecules.
- Invented a fluorescence-based assay to detect antibody-producing clones.
- Supervised small-scale protein purification to support early development of new molecules.

ROCHE MOLECULAR SYSTEMS, Bioprocessing, Branchburg, NJ

Senior Scientist, 2004

- Supervised GMP manufacturing of AmpliTaq with annual sales of over $100 million while maintaining 98% on-time-delivery rate.
- Developed purification process and completed technology transfer of G46E CS5 DNA polymerase from research to GMP manufacturing.
- Implemented AAS as Mg^{2+}/Mn^{2+} concentration determination method.

MOJAVE THERAPEUTICS INC., Protein Expression & Purification, Hawthorne, NY

Scientist, 2001- 2003

- Cloned, expressed, purified and characterized over 15 proteins in bacterial, insect, and mammalian expression systems.
- Engineered a novel cost effective protein expression and one-step purification system to produce moderate amounts of native, >92% pure, and endotoxin-free company's lead therapeutic proteins for pre-clinical studies.
- Developed a native protein purification method and optimized into an economical ($250K cheaper), efficient and GMP transferable process by employing ion exchange and affinity column purifications.
- Developed a process to achieve high yields of an antigen after refolding from inclusion bodies.
- Established release and stability tests for therapeutic proteins using various techniques including ELISA, Native/SDS-PAGE, HPLC and SEC.
- Devised and optimized *in-vitro* protein-protein interaction based assays utilizing state of the art technologies.
- Prepared CMC sections for IND submissions.
- Supervised research associates to develop a highly productive team.

BROWN UNIVERSITY, Dept. of Molecular Pharmacology, Physiology and Biotechnology, Providence, RI

Research Assistant, 1996-2001

- Discovered and characterized the interaction of Synapse Associated Proteins (SAP/PSD) with kainate receptors.
- Cloned and expressed individual domains of SAP/ kainite receptors and developed cell-based and *in-vitro* assays to identify the molecular mechanisms regulating differential interaction of kainate receptors with SAPs.
- Elucidated signaling pathways of insulin like growth factor-1 (IGF-1) for neuronal survival by discovering activation of protein kinases and calcium gated ion channels.
- Stable isotope labeling of recombinant PDZ1, SH3 and Guanylate kinase domains with ^{15}N and ^{13}C for NMR structure determinations.
- Trained and supervised research assistants.

Teaching Assistant, 1997-2001

- Taught CNS, CVS and cancer chemotherapy topics to the medical students for their pharmacology course.
- Integrally involved in the preparation and evaluation of exams.

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES, Pathology, Bethesda, MD

Research Associate, 1994-1996

- Devised a patented algorithm and semi-high throughput assays to screen two or more anti-cancer drugs for their synergistic, antagonistic or additive interactions.
- Invented retardation of cell cycle by low-dose of cancer chemotherapeutic agents and exploited the phenomenon to induce synergistic apoptosis by additional agents.

CENTRAL DRUG RESEARCH INSTITUTE, Microbiology and Drug Development, Lucknow, India

Research Trainee, 1992

- Developed a new regimen against chloroquine resistant rodent malaria utilizing fluroquinolone antibiotics (e.g. Ciprofloxacin and Norfloxacin).

EDUCATION

BROWN UNIVERSITY, Providence, RI

- **Ph.D.**, Medical Science, 2001

Dissertation: Identification and Characterization of Kainate Receptor Interaction with PSD-95/SAP90 Family proteins.

BROWN UNIVERSITY, Providence, RI

- **Sc.M.**, Medical Science, 1999
- GPA of **4.0**

Thesis: SAP90 Binds and Clusters Kainate Receptors Causing Incomplete Desensitization

B.I.T.S., Pilani, Rajasthan, India / **UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES**, Bethesda, MD

- **B. Pharmacy**, with Honors, 1994
- Ranked first in the graduate class.

Thesis: Synergistic Potentiation of Anti-cancer Drugs by Cell Cycle Retardation.

AWARDS AND HONORS

- Encore award from Centocor 2006
- Innovation Award from Johnson and Johnson 2005
- Best Presentation Award from ASPET 2000
- Pharmacia and Upjohn New England Pharmacologist Award 1999
- SmithKline Beecham Pharmacology Award 1998
- Fellowship from Brown University 1996-1997
- Fellowship from Uniformed Services University of the Health Sciences, Bethesda MD 1994-1996
- Sustained Superior Performance Award from Henry M. Jackson Foundation for the Advancement of Military Medicine 1995
- National Scholarship from Directorate of Education, Delhi, India 1990-1994
- Outstanding Academic Performance Award from Directorate of Education, Delhi, India 1989

TECHNICAL SKILLS

Molecular Biology: Cloning, PCR, RT-PCR, mutagenesis, siRNA design, DNA sequencing, hybridization, phage display, Affymetrix-GeneChip, Southern blotting, northern blotting and electrophoretic mobility shift assays (EMSA).

Biochemistry: Fermentation, bacterial, insect, and mammalian protein expression and purification, ^{15}N and ^{13}C isotope labeling, HPLC, affinity, ion-exchange and size-exclusion chromatography, capillary electrophoresis, LAL

assay, ELISA, BIACORE, fluorescence anisotropy, in-solution binding assays, radioligand bindings, *in-vitro* kinase assays, enzyme kinetics, immunoprecipitation, native/SDS-PAGE, immunoblotting, and crosslinking,.
General Cell Biology: Tissue culture (from primary and established cell lines), transfection (using cDNA and oligonucleotides), infection, protein transduction, immunohistochemistry, microscopy (light, fluorescent and confocal), Fura-2 calcium imaging, cell-surface protein labeling, FACS, cell cycle, growth inhibition (clonogenic and MTT), cytotoxicity (LDH) and apoptosis detection assays.

Considerable experience in working under GLP/GMP environment and project management.
Independent radioisotope user certification for P^{32}, S^{35} and C^{14}
Certificate course in rodent handling.

Computer skills: Proficient in use of Medline, Entrez, Blast, Vector NTI advance, Amplify, DNA Strider, GCG Online, Jellyfish, EndNote, SwissModel, Windows XP, Microsoft Office XP, MacOS X, Unix, Adobe Photoshop and HTML.

PROFESSIONAL AFFILIATION

- American Society for Pharmacology and Experimental Therapeutics (ASPET)
- Society for Neuroscience

SELECTED PATENT AND PUBLICATIONS

- Patent filed on Fluorescent Protein A/G Based Antibody Secretion Detection Assay (Filed, Mar 2007)
- Patent filed on Heat Shock Protein-Based Vaccines and Immunotherapies. PCT/US2004/010983 and US20050202033A1, and US20050214312A1 (pending).
- Patentee of "A Method of Dynamic Retardation of Cell Cycle Kinetics to Potentiate Cell Damage" US6455593B1, US6274576B1, EP835111A2, WO9701344A2, AU031314A5, AU00715527B2, AU06396096A1.
- Flechtner, J.B., Cohane, K.P., **Mehta, S.**, Slusarewicz, P., Leonard, A.K., Barber, B.H., Levey, D.L., and Andjelic, S. (2006). High-affinity interactions between peptides and Heat Shock Protein 70 augment CD8+ T lymphocyte immune responses. **J Immunol** *177*, 1017–1027.
- Piserchio, A., Pellegrini M., **Mehta, S.**, Blackman, S., Garcia, E., Marshall, J. and Dale F. Mierke (2002). The PDZ1 domain of SAP90: Characterization of structure and binding. **J Biol Chem** *277*, 6967-6973.
- **Mehta, S.**, Wu, H., Garner, C. C. and Marshall, J. (2001). Molecular mechanisms regulating the differential association of kainate receptor subunits with SAP90/PSD-95 and SAP97. **J Biol Chem** *276*, 16092-16099.
- **Mehta, S.**, Garcia, E. P., Blair, L. A., Wells, D. G., Shang, J., Fukushima, T., Fallon, J. R., Garner, C. C., and Marshall, J. (1998). SAP90 binds and clusters kainate receptors causing incomplete desensitization. **Neuron** *21*, 727-39.
- Blair, L. A., Bence-Hanulec, K. K., **Mehta, S.**, Franke, T., Kaplan, D., and Marshall, J. (1999). Akt-dependent potentiation of L channels by insulin-like growth factor- 1 is required for neuronal survival. **J Neurosci** *19*, 1940-51.
- Brem, A. S., Bina, R. B., **Mehta, S.**, and Marshall, J. (1999). Glucocorticoids inhibit the expression of calcium-dependent potassium channels in vascular smooth muscle. **Mol Genet Metab** *67*, 53-7.
- Rui, H., Xu, J., **Mehta, S.**, Fang, H., Williams, J., Dong, F., and Grimley, P. M. (1998). Activation of the Jak2-Stat5 signaling pathway in Nb2 lymphoma cells by an anti-apoptotic agent, aurintricarboxylic acid. **J Biol Chem** *273*, 28-32.

SELECTED ABSTRACTS AND PRESENTATIONS

- Baker, J., **Mehta, S.**, Prince, K., Slusarewicz, P., Barber, B. and Andjelic, S. (2003). Higher affinity interaction between MHC class I epitope and HSP70 augments $CD8^+$ T lymphocyte immune responses. **Keystone Symposium on Basic Aspects of Tumor Immunobiology**.
- **Mehta, S.**, Garner, C.C. and Marshall, J. (2000) Molecular mechanisms regulating the differential interaction of SAP97 with kainate receptors. **American Society for Pharmacology and Experimental Therapeutics Meeting**.
- **Mehta, S.** and Marshall, J. (1999) Differential interaction of synapse associated proteins with kainate receptors. **Gordon Research Conference on Excitatory Amino Acids and Brain Function**.
- **Mehta, S.** and Marshall, J. (1998) SAP90 binds and clusters kainate receptors via the SH3, GK and PDZ1 domains causing incomplete desensitization. **New England Pharmacologists 27th Annual Meeting**.
- Garcia, E.P., Shang, M., **Mehta, S.** and Marshall, J. (1997) Kainate receptor clustering activity of PSD-95. **27th Annual Meeting of the Society for Neuroscience.**
- **Mehta, S.**, Fields, K. and Grimley, P.M. (1996). A novel strategy of cell cycle modulation for potentiating apoptotic killing of p53-negative cells. **87th Annual Meeting of the American Association for Cancer Research**.
- **Mehta, S.** and Grimley, P.M. (1995). S-phase enhancement of p-53-independent apoptosis induced by staurosporine in human lymphoma cells. **Thirty-fifth Annual Meeting of the American Society for Cell Biology**.
- Grimley, P.M., **Mehta, S.**, Reedy, E., and Wang, C. (1995). A novel approach of cell cycle restraint potentiates the cytotoxic action of indole carbazole protein kinase inhibitors (ICI) in human lymphoma cells. **37th Annual Meeting of American Society of Hematology**.
- Wang, C., **Mehta, S.**, Stass, S. and Grimley, P.M. (1995). A novel approach to cell cycle kinetic modulation potentiates cytotoxicity in acute myeloblastic leukemia (AML) using low dose hydroxyurea (HU). **37th Annual Meeting of American Society of Hematology**.

NEW ENGLAND PEPTIDE, INC.

New Javelin

(SJAV 1) **Certificate of Analysis**

Sequence: H2N-NLLRLTGW-OH			
Peptide Name	**Lot #**	**Milligrams**	**Date**
	268-41/42	5.4	

1.0 Product Name and Formulation: Bioactive Peptide. Final product is supplied as a lyophilized powder containing traces of trifluoroacetate salts. If other list:_____

2.0 Quality Control Specifications:

QC Test	QC Specifications	Results	Approval/Initials
Purity	Performed by HPLC, must be > 90+ % by percent area on standard HPLC gradient (see column QC.)	99.9%	PP
Mass Identification	Performed MALDI-TOF DE mass spectral analysis, mass to be within 0.1% of exact molecular weight: 971	972	PP
Concentration (if required)	Spectrophotometric analysis (A280) or Amino Acid Analysis confirming net peptide content.		

3.0 Notes: soluble in H_2O, add AcN if needed

4.0 Indirect Materials:

Description	Source	Part Number	Lot #
Acetonitrile	J.T. Baker	9017-03	821
Water	J.T. Baker	4218-03	e63
Trifluoroacetic acid	American Bioanalytical	AB2010	3022
HPLC Column	YMC	C18	aq23
Dimethyl Formamide	J.T. Baker	9344-13	5b02
Alpha cyano cinnamic acid	SIGMA	C2020	68H3685

Quality Control Detail Information

5.0 Quality Control Equipment

HPLC – Gilson HPLC
Mass Spec - PE Biosystems Voyager DE MALDI-TOF
Spectrophotometer - Milton Roy Spectronic 21DU

6.0 Reagent Preparation:

HPLC Buffers: **A:** HPLC grade Water with 0.1%TFA (If other List:_____)
B: Acetonitrile with 0.08% TFA

Mass Spectral Matrix: 10mgs alpha cyano cinnamic acid in 500ul A, 500ul B
Dissolve in eppindorf, spin down pellet, decant and use supernatant

7.0 Quality Control Protocol:

A. HPLC Analysis
Gradient: as shown
Injection: approximately 1mg/ml peptide in HPLC grade water, 100ul (100ug) per injection
Percent purity based on peak area

B. Mass Spec
spot 1ul matrix with 1ul 1mg/ml peptide solution
let air dry, run sample
different voltages, ion charges, and setting shown on Mass Spectral Analysis
Mass to be within 0.1% of exact molecular weight

C. Spectrophotometric Analysis (If required)
after final lyophilization weigh out 2 vials of peptide (from 1-5mgs)
dissolve in 1-5ml HPLC water at approximately 1.0mg/ml
read abs of each sample at 280nm
calculate concentration and net peptide content

D. AAA Analysis (If required)
sample must be within 10% for each amino acid
sequence ratios must be confirmed by analysis
Note: cysteine and tryptophan residues are destroyed during analysis, number will not be accurate

8.0 Reassay Interval of Stored Samples:

Every two years or each time a lot is aliquotted from bulk storage.

8.01 Stability Information:

Lyophilized peptides generally have excellent stabilities, often showing little or no degradation after a few years at -20 deg C. Long term storage (>1year) should be at -80 deg C desiccated, medium term storage (1-12 months) should be at -20 deg C desiccated, short term storage (<1 month) may be at 4 deg C.

NEW ENGLAND PEPTIDE, INC.

Method: LDE1001
Accelerating Voltage: 25000
Grid Voltage: 90.000 %
Guide Wire Voltage: 0.100 %
Delay: 200 ON
Sample: 04

Laser : 2270
Scans Averaged: 6
Pressure: 1.64e-07
Low Mass Gate: OFF
Negative Ions: OFF

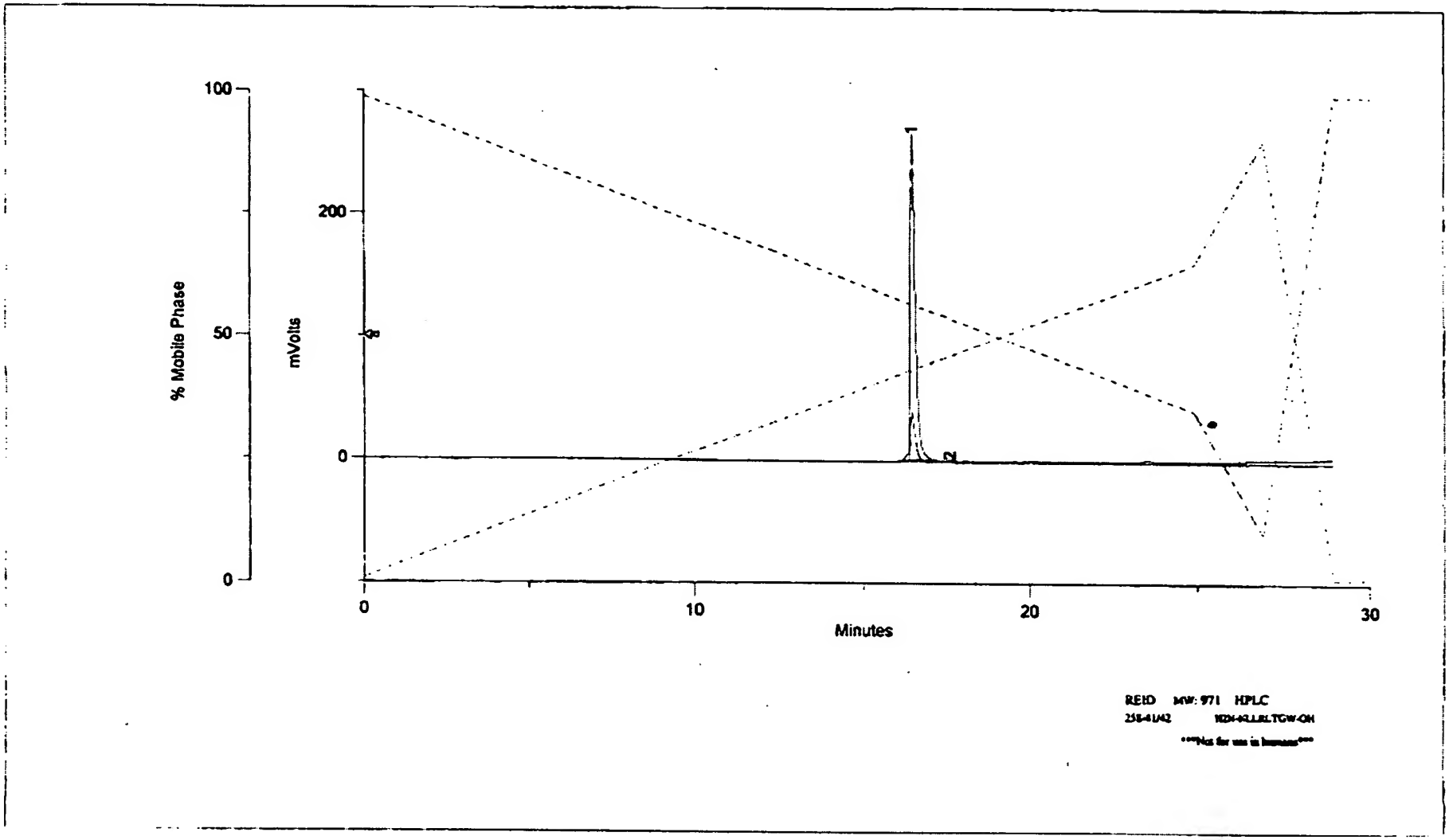
Savitsky-Golay Order = 2 Points = 19

4000

(M+H)

REID MW: 971 Mass Spec

Report



REID MW: 971 HPLC

NEW ENGLAND PEPTIDE, INC.

New Javier
(SJAV 2) = MJAV

Certificate of Analysis

Sequence:	H2N-FYQLALTW-OH

Peptide Name	Lot #	Milligrams	Date
	258-43/44	6.9	

1.0 Product Name and Formulation: Bioactive Peptide. Final product is supplied as a lyophilized powder containing traces of trifluoroacetate salts. If other list:_____

2.0 Quality Control Specifications:

QC Test	QC Specifications	Results	Approval/Initials
Purity	Performed by HPLC, must be > 90+ % by percent area on standard HPLC gradient (see column QC.)	98.3%	PP
Mass Identification	Performed MALDI-TOF DE mass spectral analysis, mass to be within 0.1% of exact molecular weight: 1040	1040	PP
Concentration (if required)	Spectrophotometric analysis (A280) or Amino Acid Analysis confirming net peptide content.		

3.0 Notes: soluble in H_2O, add AcN if needed

4.0 Indirect Materials:

Description	Source	Part Number	Lot #
Acetonitrile	J.T. Baker	9017-03	821
Water	J.T. Baker	4218-03	c63
Trifluoroacetic acid	American Bioanalytical	AB2010	3022
HPLC Column	YMC	C18	aq19
Dimethyl Formamide	J.T. Baker	9344-13	5b02
Alpha cyano cinnamic acid	SIGMA	C2020	68H3685

Quality Control Detail Information

5.0 Quality Control Equipment

HPLC – Gilson HPLC
Mass Spec - PE Biosystems Voyager DE MALDI-TOF
Spectrophotometer - Milton Roy Spectronic 21DU

6.0 Reagent Preparation:

HPLC Buffers: A: HPLC grade Water with 0.1%TFA (If other List:_____)
B: Acetonitrile with 0.08% TFA

Mass Spectral Matrix: 10mgs alpha cyano cinnamic acid in 500ul A, 500ul B
Dissolve in eppindorf, spin down pellet, decant and use supernatant

7.0 Quality Control Protocol:

A. HPLC Analysis
Gradient: as shown
Injection: approximately 1mg/ml peptide in HPLC grade water, 100ul (100ug) per injection
Percent purity based on peak area

B. Mass Spec
spot 1ul matrix with 1ul 1mg/ml peptide solution
let air dry, run sample
different voltages, ion charges, and setting shown on Mass Spectral Analysis
Mass to be within 0.1% of exact molecular weight

C. Spectrophotometric Analysis (If required)
after final lyophilization weigh out 2 vials of peptide (from 1-5mgs)
dissolve in 1-5ml HPLC water at approximately 1.0mg/ml
read abs of each sample at 280nm
calculate concentration and net peptide content

D. AAA Analysis (If required)
sample must be within 10% for each amino acid
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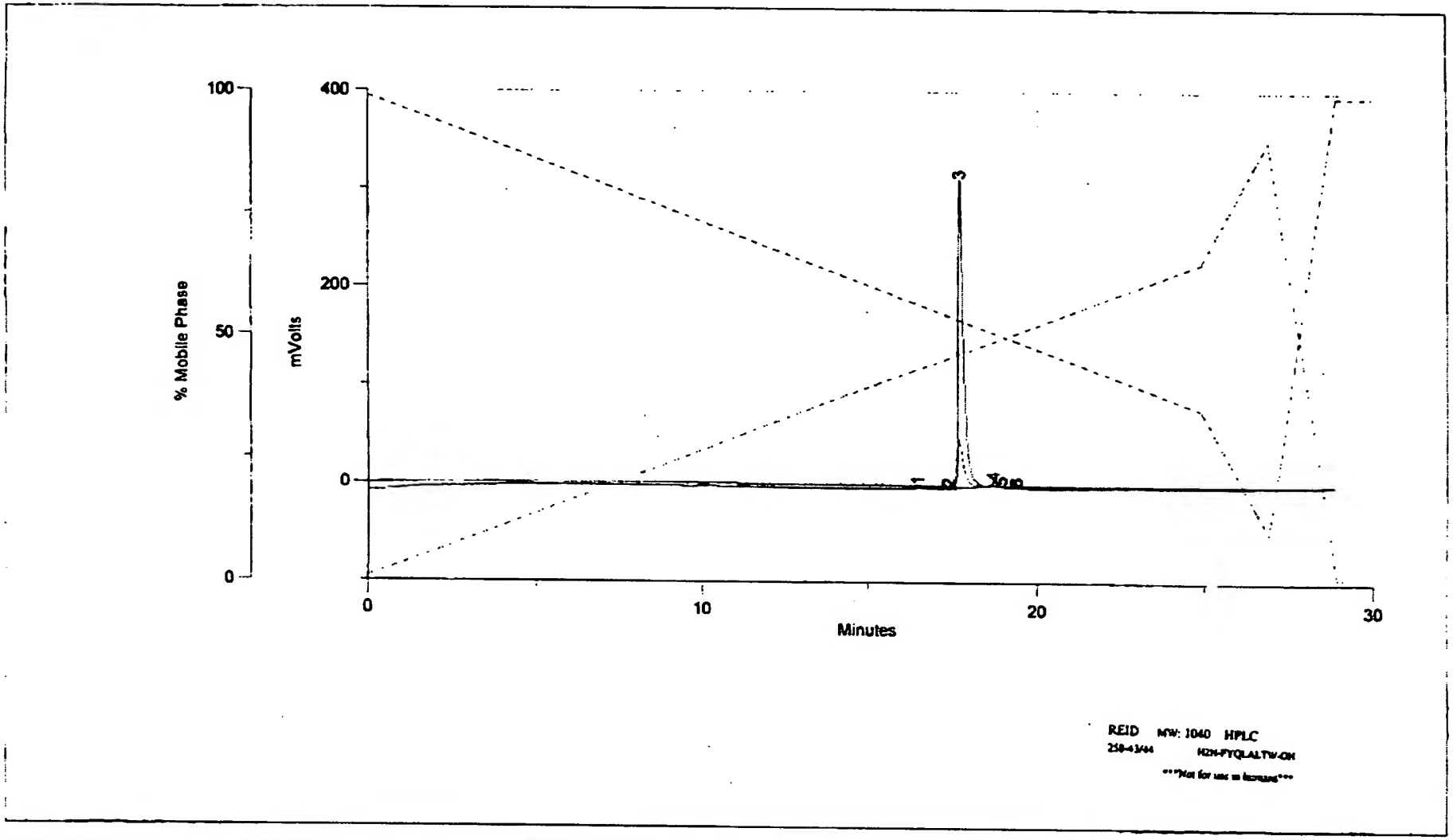

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4.00	2	17.40	0.13	7568.75								
4.00	3	17.70	98.34	5950929.50								
4.00	4	18.71	0.81	48870.38								
4.00	5	18.95	0.09	5342.92								

4.00	6	19.40	0.13	7829.15								

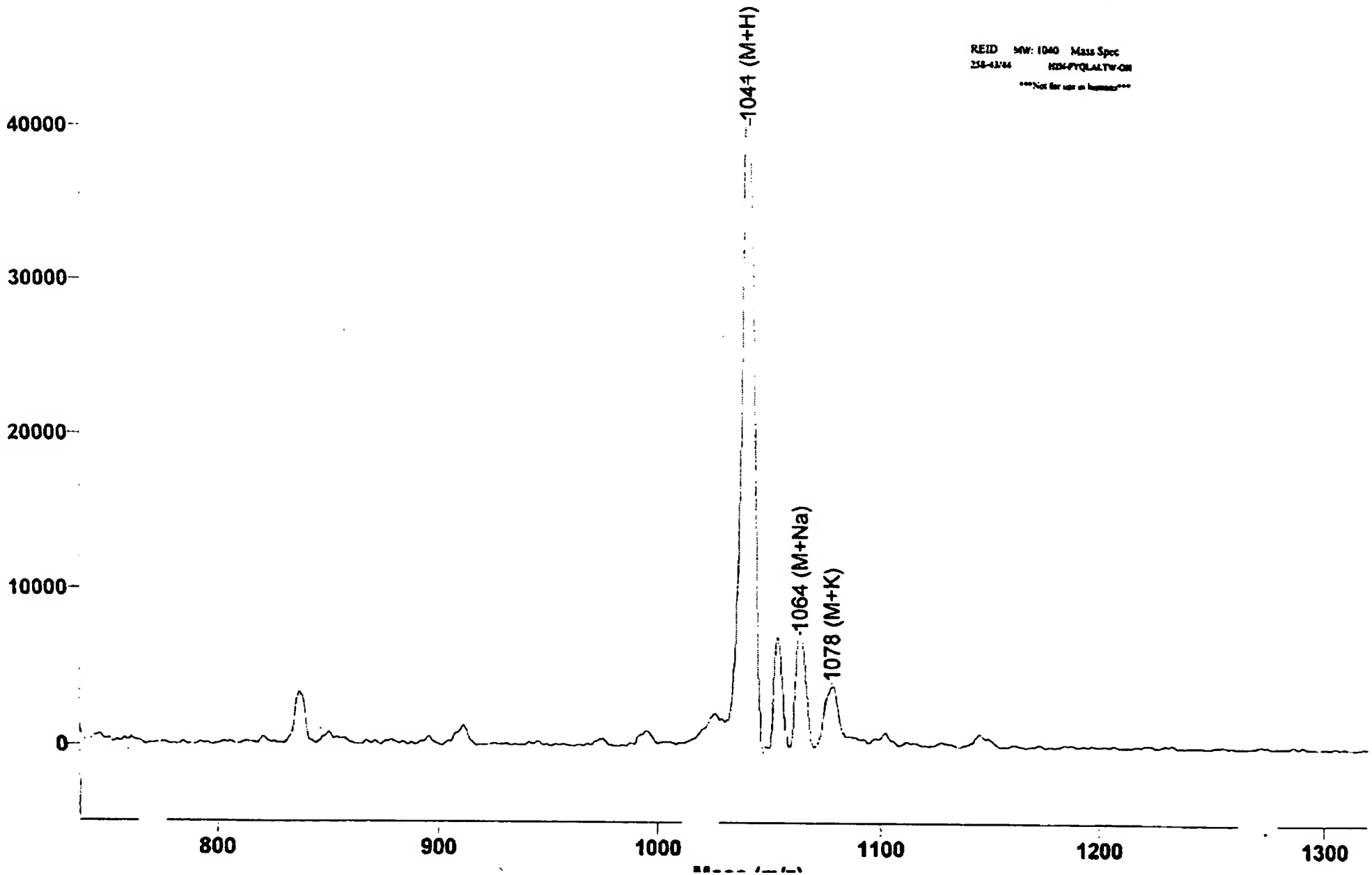
NEW ENGLAND PEPTIDE, INC.

Method: LDE1001
Accelerating Voltage: 25000
Grid Voltage: 90.000 %
Guide Wire Voltage: 0.100 %
Delay: 200 ON
Sample: 03

Laser : 2270
Scans Averaged: 8
Pressure: 1.69e-07
Low Mass Gate: OFF
Negative Ions: OFF

Savitsky-Golay Order = 2 Points = 19

REID MW: 1040 Mass Spec
258-43/44 H[DL-PYQLALTW-OH
Not for use in humans



NEW ENGLAND PEPTIDE, INC.

J3

New Injection

Certificate of Analysis

Sequence: H2N-RKLFFNLRW-OH

Peptide Name	Lot #	Milligrams	Date
	258-34/35	5.5	

1.0 Product Name and Formulation: Bioactive Peptide. Final product is supplied as a lyophilized powder containing traces of trifluoroacetate salts. If other list:_____

2.0 Quality Control Specifications:

QC Test	QC Specifications	Results	Approval/Initials
Purity	Performed by HPLC, must be > 90+ % by percent area on standard HPLC gradient (see column QC.)	99.99%	PP
Mass Identification	Performed MALDI-TOF DE mass spectral analysis, mass to be within 0.1% of exact molecular weight: 1278	1278	PP
Concentration (if required)	Spectrophotometric analysis (A280) or Amino Acid Analysis confirming net peptide content.		

3.0 Notes: soluble in H_2O, add AcN if needed

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HPLC Column	YMC	C18	aq20
Dimethyl Formamide	J.T. Baker	9344-13	5b02
Alpha cyano cinnamic acid	SIGMA	C2020	68H3685

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Mass Spec - PE Biosystems Voyager DE MALDI-TOF
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Percent purity based on peak area

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different voltages, ion charges, and setting shown on Mass Spectral Analysis
Mass to be within 0.1% of exact molecular weight

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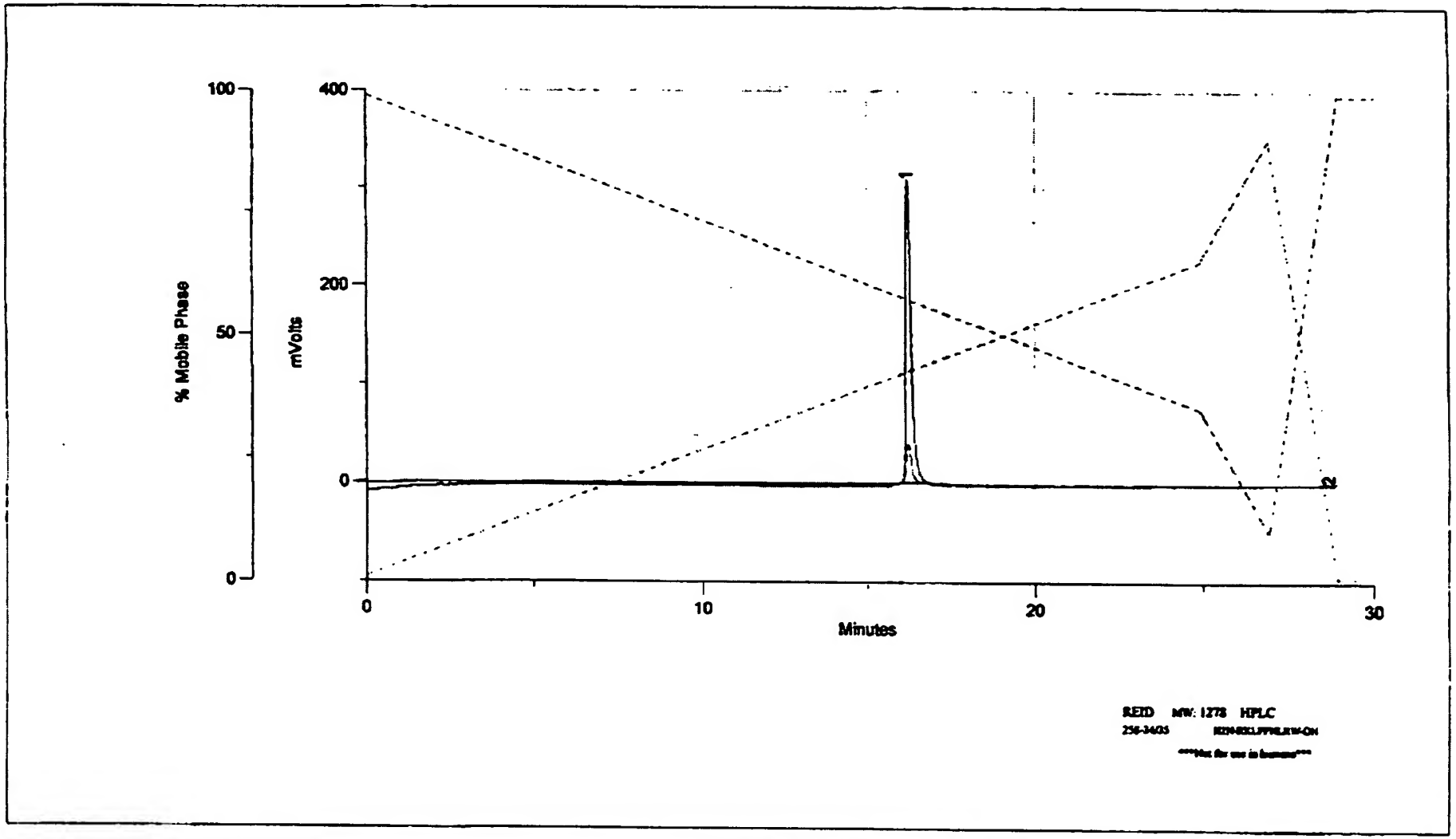

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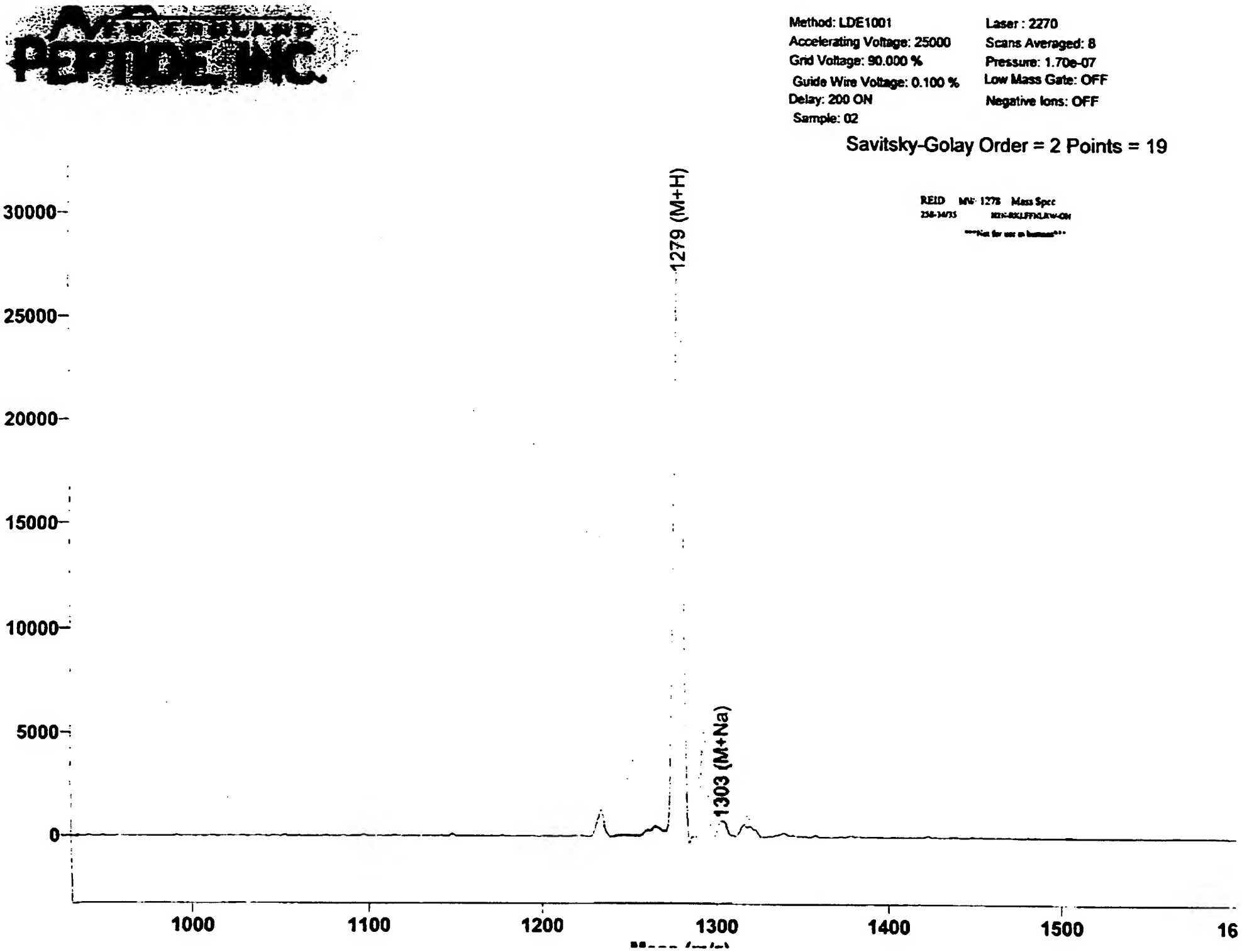
3.00	2	28.66	0.01	773.74								


Method: LDE1001
Accelerating Voltage: 25000
Grid Voltage: 90.000 %
Guide Wire Voltage: 0.100 %
Delay: 200 ON
Sample: 02
Laser : 2270
Scans Averaged: 8
Pressure: 1.70e-07
Low Mass Gate: OFF
Negative Ions: OFF
Savitsky-Golay Order = 2 Points = 19
1279 (M+H)
1303 (M+Na)
30000
25000
20000
15000
10000
5000
0
1000
1100
1200
1300
1400
1500
16

rom Page No.

OVA chicken egg 43000D

OVA 20mg/ml 0.3ml 6mg - 0.14μM
on 2.5μM according
18 lysines per OVA molecule
14mM

DSS 23.3mg/ml 0.3ml 7mg 8.2μM
DMSO

DSS - 366.4

S-Jan 1 - 972 D - 417μl
1.05 mg

Jan GSG - 1344 D - 33mg/ml
1.1 g

Activation

OVA was dissolved in H2O 25μl 0.2M MES pH 8.0
added to adjust pH to ~7÷7.5

DSS added & solution became cloudy

Incubation for 15', then 20μl 0.2M MES/2mM EDTA pH 5.0
added - (to stabilize ester bond)

Solution was centrifuged & loaded on P6 Biogel 1.5×88cm
column (V=16ml)

Purification

Column is equilibrated with 0.005M MES/2mM EDTA pH 5.0

The first peak was collected - 1.5ml 2.3mg/ml 3.44mg
+ 0.5ml 0.53mg/ml

Conjugation

0.75ml OVA DSS + 36μl Jan G-SG
1.72mg (.04μM / .72μM) + 1.19mg (0.9μM) - precipitation, 100μl DMSO
added → clear
+ 90μl 0.2M NaPh pH 8.0 → pH 7.0

0.52ml OVA DSS + 0.03ml S-Jan 1 -
1.2mg (.028μM / .5μM) + 1.25mg (1.29μM) - pH was adjusted with 0.5N
NaOH to reduce the volume
IV: 1.05ml - clear

Incubation for 1hr 30' — RT

The conjugation was stopped by 6Cysine 100mg/ml
(10μl into each mixture)

The conjugates were purified using P6 Biogel (1.5×4cm)

To Page No. 58

Witnessed & Understood by me, Date 4.4.L

Invented by AK Date

Recorded by AK

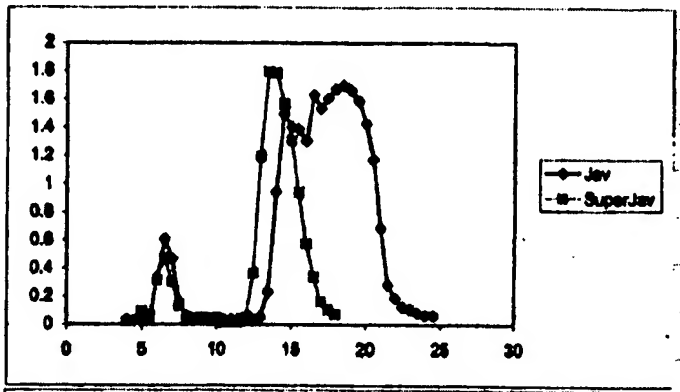
From Page No. 57

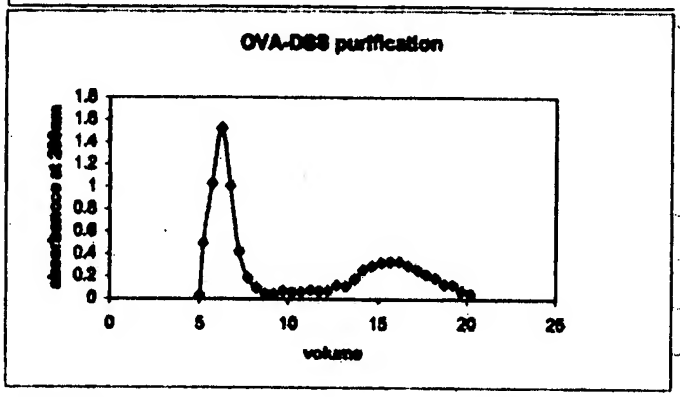
OVA-DSS

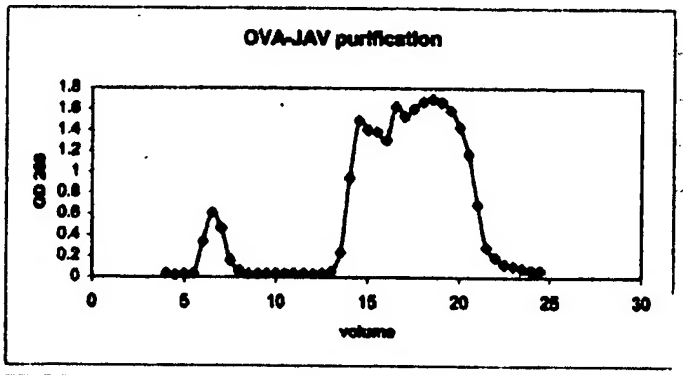
volume	OD280	fr. volume
5	0.033	5
5.2	0.498	0.2
5.7	1.029	0.5
6.2	1.525	0.5
6.7	1.007	0.5
7.2	0.423	0.5
7.7	0.186	0.5
8.2	0.097	0.5
8.7	0.043	0.5
9.2	0.04	0.5
9.7	0.07	0.5
10.2	0.058	0.5
10.7	0.082	0.5
11.2	0.079	0.5
11.7	0.069	0.5
12.2	0.076	0.5
12.7	0.122	0.5
13.2	0.114	0.5
13.7	0.178	0.5
14.2	0.266	0.5
14.7	0.296	0.5
15.2	0.334	0.5
15.7	0.33	0.5
16.2	0.331	0.5
16.7	0.294	0.5
17.2	0.256	0.5
17.7	0.217	0.5
18.2	0.184	0.5
18.7	0.127	0.5
19.2	0.123	0.5
19.7	0.089	0.5
20.2	0.048	0.5

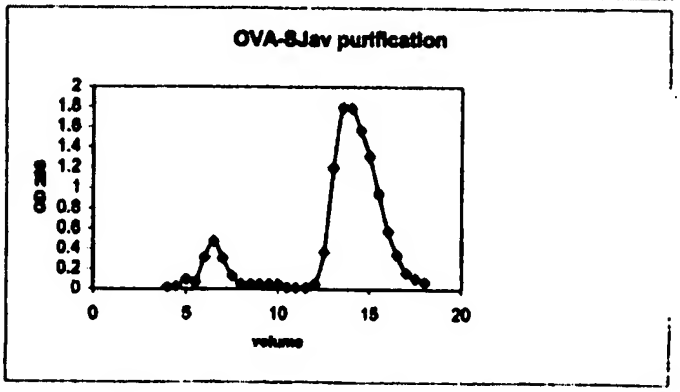
OVA-JAV

volume	OD280	fr.volume
4	0.037	4
4.5	0.03	0.5
5	0.03	0.5
5.5	0.037	0.5
6	0.336	0.5
6.5	0.609	0.5
7	0.466	0.5
7.5	0.161	0.5
8	0.069	0.5
8.5	0.03	0.5
9	0.032	0.5
9.5	0.037	0.5
10	0.037	0.5
10.5	0.037	0.5
11	0.037	0.5
11.5	0.037	0.5
12	0.037	0.5
12.5	0.037	0.5
13	0.064	0.5
13.5	0.233	0.5
14	0.944	0.5
14.5	1.492	0.5
15	1.404	0.5
15.5	1.368	0.5
16	1.304	0.5
16.5	1.629	0.5
17	1.534	0.5
17.5	1.609	0.5
18	1.573	0.5
18.5	1.697	0.5
19	1.695	0.5
19.5	1.586	0.5
20	1.43	0.5
20.5	1.173	0.5
21	0.839	0.5
21.5	0.284	0.5
22	0.187	0.5
22.5	0.123	0.5
23	0.106	0.5
23.5	0.084	0.5
24	0.057	0.5
24.5	0.055	0.5









OVA-SJAV

volume	OD280	fr.volume
4	0.02	4
4.5	0.029	0.5
5	0.057	0.5
5.5	0.057	0.5
6	0.316	0.5
6.5	0.475	0.5
7	0.303	0.5
7.5	0.13	0.5
8	0.049	0.5
8.5	0.049	0.5
9	0.049	0.5
9.5	0.049	0.5
10	0.049	0.5
10.5	0.014	0.5
11	0.014	0.5
11.5	0.014	0.5
12	0.063	0.5
12.5	0.368	0.5
13	1.197	0.5
13.5	1.794	0.5
14	1.784	0.5
14.5	1.57	0.5
15	1.307	0.5
15.5	0.943	0.5
16	0.576	0.5
16.5	0.341	0.5
17	0.162	0.5
17.5	0.107	0.5
18	0.073	0.5

SV

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Witnessed & Understood by me, Date 4/

Invented by A K Date

Recorded by A K

From Page No. new

Maleimide S. activated OVA with DSP

10 mg/ml OVA, 1:10 M excess of cr/c pH 9.

After purification (P6 Bio-gel) Fr N° 15 was taken

Fr 15 — 0,5 ml OD 0,15 1:10 → 1,65 mg/ml → 0,82 mg → 0,02 mmoles

OVA 4,9 mg in 0,245 ml 10 mM NaPB / 0,15 M NaCl pH 6,5 →

→ 0,114 mmoles

OVA & OVA-DSP were reduced with 1M DTT

OVA — 40x molar excess → 4,6 ml 1M DTT 40' RT

OVA-DSP - 100x molar excess → 2 mmoles, 1 µl per 0,25 ml OVA DSP

Protein was purified from DTT excess using PD-10 column

~ 0,244 ml of OVA loaded, then 2,25 ml (+2,5 ml) of NaPB buffer pH 6,5 → flow through

Elution → Fr 1 - 0,4 ml, Fr 2 ... - 0,5 ml

	ε_{280} OVA DSP-SH	ε_{280} OVA-SH	
Fr 1	0,005	0,013	OVA-DSP-SH ~ 0,34 mg/ml ~ 8 µMoles
Fr 2	0,013	0,218	OVA-SH ~ 4,15 mg/ml ~ 93 µMoles
Fr 3	0,256	2,103 → 1:10 0,340	Fr 3 P2 (1:10) - 0,15 mg/ml 78 µM
Fr 4	0,283	2,010	
Fr 5	0,069	0,437	

SJ 2 peptide Fr 17 1:10 E - 0,604 (2,338 w/o mAcn)

Fr 18 1:10 E - 0,233 (1,849 w/o mAcn) ~ 0,043 mg/ml ($\frac{1040}{1550}$) not true

The determination of SJ2 concentration is not correct

Based on the P2 purification we assumed, that we have ~ 80 µM SJ2 concentration & performed SJ2-SMPB & P2-SMPB hot conjugations with OVA DSP-SH & OVA-SH, using different molar ratios

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Witnessed & Understood by me,

Date 12

Invented by

Date

Recorded by

Project No.

Book No. 44 TITLE Hot P2 conjugation with OVA-DSP-SH & OVA-SH

From Page No. 73 Hot P2 was activated & purified by Gel-P, Fraction N° 3, with highest concents & OD was also so,

Fr3 – 0,18 mg/ml 78 μM

OVA DSP-SH – 0,34 g/ml 80 μM

OVA-SH – 4,1 g/ml 93 μM

OVA - P2 conjugation

Molar ratio	OVAact μl	P2 μl	OVA μl	P2 μl
1:10	60	60	6	60
1:2	80	40	10	50
1:2	100	20	20	40

OVA - SJ2 conjugation

OVAact μl	SJ2	OVA	SJ2
60	60		
80	40	10	50
50	20	20	40

Conjugation o/N 4°C

Jason performed inhibition assay with SJ2 conjugates using 1:2 OVA-SH-ISL conjugates. That's why they were not analysed by HPLC

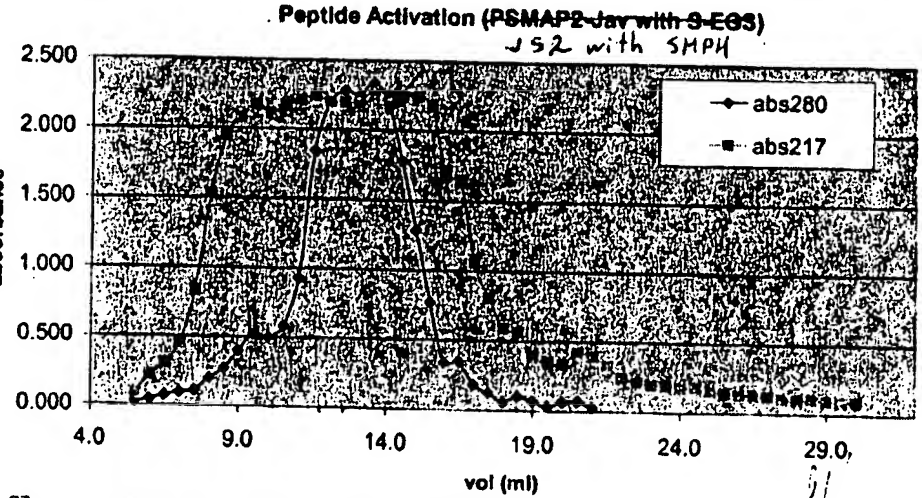
In case of OVAact SJ2 was diluted 2 times 3 times & 6 times

In case of OVA SJ2 was diluted by 15% & 33%

HPLC analysis of the conjugates & Fr 13 & Fr 17 – JSA-SMPH was made

Peptide Activation (SJ2 (FYQLALTW) with SMPH)

Fract #	vol (ml)	abs 280	abs 217	[mg/ml]	mass
	5.0				
1	5.5	0.025	0.080	0.002	0.001
2	6.0	0.046	0.218	0.003	0.001
3	6.5	0.075	0.301	0.005	0.002
4	7.0	0.097	0.449	0.006	0.003
5	7.5	0.111	0.830	0.007	0.003
6	8.0	0.199	1.523	0.013	0.006
7	8.5	0.269	1.944	0.017	0.008
8	9.0	0.395	2.082	0.025	0.012
9	9.5	0.518	2.173	0.033	0.016
10	10.0	0.495	2.110	0.031	0.016
11	10.5	0.580	2.173	0.037	0.018
12	11.0	0.924	2.207	0.058	0.029
13	11.5	1.849	2.245	0.117	0.058
14	12.0	2.197	2.245	0.138	0.069
15	12.5	2.282	2.245	0.144	0.072
16	13.0	2.252	2.245	0.142	0.071
17	13.5	2.338	2.286	0.147	0.074
18	14.0	2.187	2.245	0.138	0.069
19	14.5	1.784	2.245	0.112	0.056
20	15.0	1.290	2.245	0.081	0.041
21	15.5	0.769	2.173	0.048	0.024
22	16.0	0.339	1.684	0.021	0.011
23	16.5	0.347	1.643	0.022	0.011
24	17.0	0.182	1.059	0.011	0.006
25	17.5	0.126	0.806	0.008	0.004
26	18.0	0.062	0.589	0.004	0.002
27	18.5	0.102	0.559	0.006	0.003
28	19.0	0.070	0.397	0.004	0.002
29	19.5	0.032	0.343	0.002	0.001
30	20.0	0.066	0.336	0.004	0.002
31	20.5	0.071	0.423	0.004	0.002
32	21.0	0.033	0.392	0.002	0.001
33	21.5	0.026	0.329	0.002	0.001
34	22.0	0.019	0.238	0.001	0.001
35	22.5	0.020	0.213	0.001	0.001



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Invented by

Date

Recorded by

From Page No. 74

SAM NO	POS	TIME MIN	H#	3H CPM	3H %ERROR	3H DPM	3H EFF-1	LUMEX %	ELAPSED TIME	
1	** 1	1.00	76.9	234429.0	0.41	467763.6	50.12	0.00	1.44	
2	**-2	1.00	76.1	239467.0	0.41	475903.8	50.32	0.00	3.35	
3	**-3	1.00	76.3	317228.0	0.36	631070.6	50.27	0.00	5.12	
4	**-4	1.00	75.5	283692.0	0.38	562180.8	50.46	0.00	6.85	
5	**-5	1.00	78.7	338720.0	0.34	681785.6	49.68	0.00	8.63	
6	**-6	1.00	74.9	337270.0	0.34	666469.2	50.61	0.00	10.38	
7	**-7	1.00	77.2	347009.0	0.34	693194.2	50.06	0.00	12.17	
8	**-8	1.00	74.8	342530.0	0.41	479038.5	50.63	0.00	13.89	
9	**-9	1.00	76.4	324158.0	0.35	645292.8	50.23	0.00	15.66	
10	**-10	INVALID SAMPLE COUNT:				H# ABORT: COUNT RATE TOO LOW				
11	**-11	1.00	77.7	150.00	16.33	300.37	49.94	0.03	17.97	3 - HSP
12	**-12	1.00	75.7	153.00	16.17	303.47	50.42	0.03	19.58	
13	**-13	1.00	77.9	806.00	7.04	1619.71	49.89	0.01	21.17	3 + HSP
14	**-14	1.00	76.1	838.00	6.91	1665.58	50.31	0.00	22.71	
15	**-15	1.00	76.6	176.00	15.05	350.69	50.19	0.02	24.30	3 Fraction 13 neat
16	**-16	1.00	75.6	162.00	15.71	321.24	50.42	0.02	25.94	
17	**-17	1.00	76.3	583.00	8.27	1163.88	50.26	0.01	27.54	3 1:6 dilution Fraction 13
18	**-18	1.00	75.7	871.00	6.78	1728.21	50.40	0.01	29.17	
19	**-1	1.00	76.0	1005.00	6.31	1996.46	50.34	0.00	30.82	3 1:3 a
20	**-2	1.00	75.1	1178.00	5.83	2329.62	50.57	0.01	32.50	
21	**-3	1.00	77.2	1289.00	5.61	2535.88	50.04	0.00	34.11	3 1:5 a
22	**-4	1.00	74.3	1102.00	6.02	2171.77	50.74	0.00	35.73	
23	**-5	1.00	76.5	1142.00	5.91	2281.64	50.23	0.00	37.38	3 1:10 a
24	**-6	1.00	75.9	1110.00	6.00	2203.69	50.37	0.00	38.95	
25	**-7	1.00	77.6	477.00	9.16	954.89	49.95	0.01	40.57	3 1:2
26	**-8	1.00	75.3	880.00	6.74	1742.04	50.51	0.00	42.18	
27	**-9	1.00	76.5	1240.00	5.68	2469.17	50.22	0.00	43.78	3 1:5
28	**-10	1.00	75.2	1186.00	5.81	2347.45	50.52	0.00	45.41	

George showed

HPLC analysis shows 2 peaks of the purified JS2-SH2H1 (Jason performed monitoring of the activation time of JS2 & quantity of the cross linker in the conjugation mixture — activated peptide has 1 peak

Question: what had happened during JS2-SH2H1 purification, or storage, if two peptide peaks appeared

Calculation of the JS2-SH2H peak area document not the decrease in the conjugation mixture! So there is conjugation, but no inhibition

	pk1	pk2 ← area		pk1	pk2	
Fr 13	$373 \cdot 10^3$	$163 \cdot 10^3$				
1:10	$258 \cdot 10^3$	$70 \cdot 10^3$	1:5	254000	410000	1:5 ←
1:5	$131 \cdot 10^3$	$41 \cdot 10^3$	← 160000/34000			
1:2	$65 \cdot 10^3$	$38 \cdot 10^3$				

DNA-DSP2

To Page No.

Witnessed & Understood by me,	Date AL	Invented by JK	Date
		Recorded by JK	

Michael Yamin

From: Sunil Metha

Sent:

To: Michael Yamin; Mee Hoe

Subject: S-Javs

Dear Michael and Mee: Here is the information about the S-Javs. If you have any questions, please let me know.

Thanks

Name	Sequence	Kd (μM) for KHSP70	Originally Checked for	Reference
S-Jav1	NLLRLTGW*	1.8	DnaK/Hsc70	J. Mol. Biol. (1994) **241**, 133-135; J. Mol. Biol. (1994) **235**, 848-854
S-Jav2	FYQLALTW*	6.0	DnaK/Hsc70	J. Biol. Chem. (1994) **269**, 30470-30478
S-Jav3	RKLFFNLRW*	23.0	DnaK	J. Mol. Biol. (1996) **256**, 829-837
S-Jav4	KLLLLLLW*	Solublization problems	Designed	Based on the predicted consensus sequence.
GSG-Jav	GSGHWDFAWPW	120	Bip	Mojave Therapeutics; Cell (1993) **75**, 717-728

tryptophan was added at the C-terminus to facilitate the quantitation of these peptides.

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